

Amendment to ÄKTAcrossflow User Manual Edition AB

System sanitization (Updated method and instruction)

If you want to be sure that the system is clean, for example when a new filter is used or a process is changed, it is recommended to perform a sanitization of the system.

If an air filter is connected to the reservoir, remove the filter. Connect the tubing included in the "Reservoir cleaning kit" 11-0033-86 at the reservoir top. Place the tubing in waste.

A purge step is optional in the method which includes a pretreatment of the retentate valve block with water and ethanol. Purge with water removes solid substances mechanically. The following purge with 70% ethanol removes air bubbles of micro size. The pretreatment of the retentate valve block improves the result of the system sanitization. It is therefore recommended to include Purge in the method when the purpose is to sanitize the system. Instructions on how to perform the purge are included in the method as pop-up dialogs.

The stirrer should be present in the reservoir during sanitization. However, it should be replaced with a new aseptic one after the sanitization.

Note: *To make sure that there is no sample left in the system before performing the sanitization, run the System sanitization method with Milli-Q water, or equivalent, to rinse the system.*

Note: *The System sanitization method can also be used to pH neutralize the system solutions after sanitization and to exchange the solution in the system to an appropriate storage solution, for example 20% ethanol.*

Sanitization of the pump piston rinsing system

When performing system sanitization, the pump piston rinsing system should be sanitized by using 1 M NaOH as liquid in the rinsing system bottles.

Sanitization of reservoir float

The reservoir float has to be sanitized separately. Remove it from the reservoir before system sanitization. The float can be autoclaved, or equivalent.

Precautions

CAUTION! Instead of a filter, use a “three way connector” (18-1170-59). Filters are not compatible with the used flows and pressures in the System sanitization method.

Replace the pH electrode with a dummy electrode.

Remove the reservoir float.



WARNING! The reservoir is over-filled during the sanitization. It is important to have tubing (11-0033-86) to waste. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.



Creating a method

- 1 In the **Method Editor** of UNICORN, open the Method Wizard.
- 2 Select the System.
- 3 In the **Basic settings** dialog, select System Sanitization as method.

Basic Settings
Method

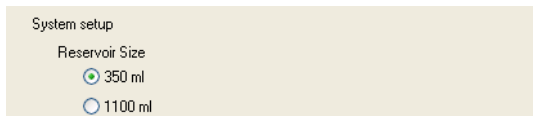
- 4 Enter Recirculation time. A minimum recirculation time of 30 min is recommended.
- 5 Select to Purge with 70% ethanol (i.e., purge with water and ethanol).

Note: *It is not necessary to include a purge if the method is used for system rinsing or system storage solutions.*

Recirculation Time min (0-999999 min)

Purge with 70% EtOH ☒ Purge

- 6 Select the Reservoir used.



Note: Information on important preparations and notes before the method is run, is displayed in the **Basic Settings** dialog. All In and Outlet connections will be used.

- 7 Click **Finish**.
- 8 In the **Summary** dialog, click **Print** to print the summary. Click **Finish** to end the Method Wizard.

Note: Do not edit any variables or method instructions.

- 9 Save the method.

Preparing the System sanitization

When running a method using a sanitization solution containing sodium hydroxide (NaOH):



WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.

CAUTION! Always make sure that the system components are compatible with sodium hydroxide at the concentration, time and temperature used.

- 1 Prepare a sanitization solution which is compatible with the ÄKTAcrossflow system, for example 1 M NaOH.

Reservoir 350 ml: 5000 ml

Reservoir 1100 ml: 7000 ml

- 2 Fill the Rinsing system bottles with 1 M NaOH (2 × 200 ml).
- 3 If a purge is included in the method, prepare 300 ml Milli-Q water, or equivalent, and 100 ml 70% ethanol.

Note: Instructions on how to perform the purge are included in the method as pop-up dialogs.

- 4 Replace the filter device with a three-way connector.
- 5 Replace the pH electrode with a dummy electrode.

- 6 Remove the float from the reservoir.
- 7 If an air filter is connected to the reservoir, remove the filter.
- 8 Connect a tubing to waste. The tubing is included in the Reservoir cleaning kit (11-0033-86).

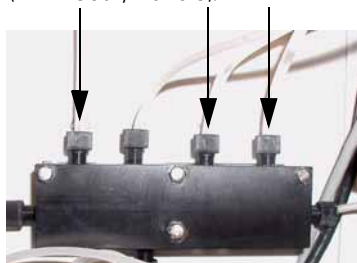


Note: To avoid siphoning back into the reservoir, keep tubing free from solution in the waste bottle.

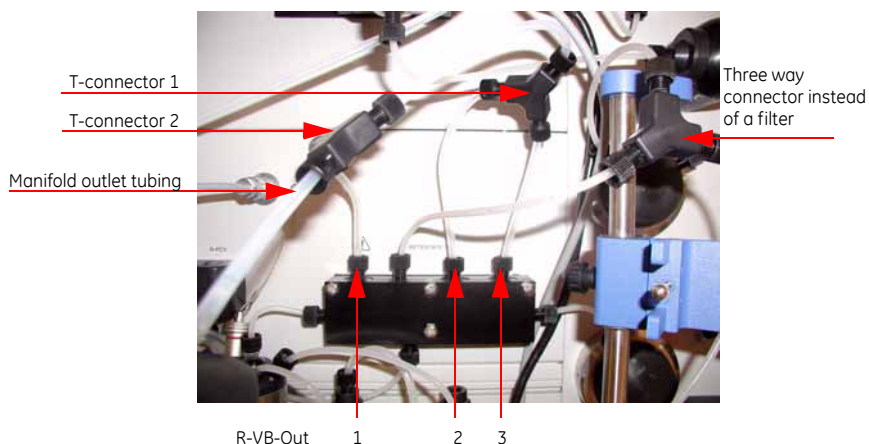
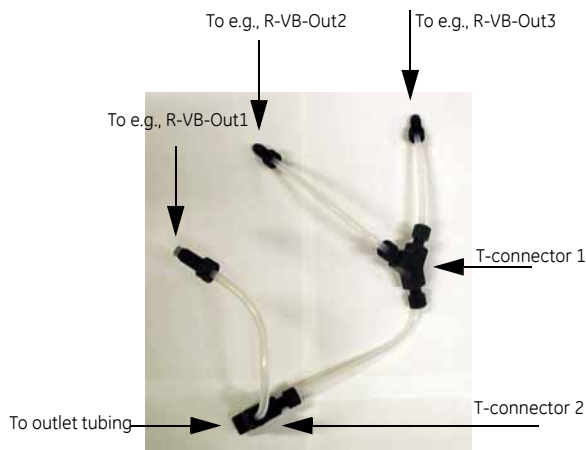


WARNING! The reservoir is over-filled during the sanitization. It is important to have tubing to waste. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.

- 9 Disconnect the outlet tubing from the Retentate valve block outlets (R-VB-Out1, 2 and 3).



- 10 Assemble the manifold included in the Reservoir cleaning kit (11-0033-86) and connect appropriate tubing ends to the Retentate valve block outlets (R-VB-Out1, 2 and 3).
- 11 Connect a tubing to the manifold outlet.



- 12 Place all inlet tubing in the prepared sanitization solution.
- 13 Place all outlet tubing and Manifold outlet tubing in a waste container.



WARNING! Ensure the waste container can hold the produced waste volume. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.

Running the System sanitization method

When running a method using a sanitization solution containing sodium hydroxide (NaOH):



WARNING! NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.

CAUTION! Always make sure that the system components are compatible with sodium hydroxide at the concentration, time and temperature used.

Before performing system sanitization, rinse the system by running the method without Purge using Milli-Q water, or equivalent.

- 1 In UNICORN, open the created System sanitization method and select **Run**.

Note: *It is also possible to run System sanitization as an Instant Run, see ÄKTAcrossflow User Manual.*

When Purge is included in the method a number of pop-up dialogs will be displayed and instruct how to perform the purge procedure. To continue to next instruction, click **Continue**.

- 2 Pour 300 ml water into the reservoir.
- 3 Check that the manifold is connected to the Retentate valve block.
- 4 Open the reservoir lid and place the outlet tubing of the manifold into the reservoir.

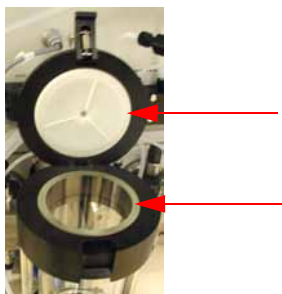


- 5 After approximately 20 minutes of purging with water, a new dialog will be displayed. Move the manifold outlet to waste.
- 6 After the reservoir has been emptied to waste, pour 100 ml of 70% ethanol into the reservoir.

- 7 Place the outlet tubing of the manifold into the reservoir.

Note: Clean the outer surface of manifold outlet tubing with 70% ethanol before placing it into the reservoir.

- 8 After approximately 10 minutes of purging with ethanol, a new dialog will be displayed. Move the manifold outlet to waste.
- 9 After the reservoir has been emptied to waste, clean with 70% ethanol the surfaces of the reservoir and the lid which will be in contact when the lid is closed.



- 10 Close the reservoir lid.
- 11 Check that the outlet tubing from reservoir top is placed in waste.

Note: To avoid siphoning back into the reservoir, keep tubing free from solution in the waste bottle.



WARNING! Ensure the waste container can hold the produced waste volume. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.



WARNING! The reservoir is over-filled during the sanitization. It is important to have tubing to waste. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves and protective clothing.

- 12 In the "Close the reservoir" dialog, click **Continue**.

The system will now be sanitized with NaOH.

- 13 Sanitize the previous disconnected R-VB-outlet tubing separately in 1M NaOH.

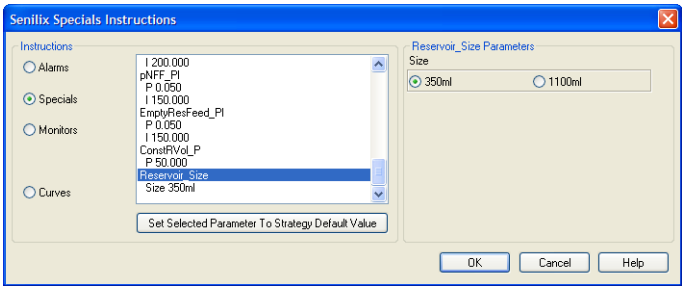
Completing sanitization procedure

- 1 Empty the system rinsing bottles and fill them with ordinary rinsing solution, 20% ethanol in 10 mM NaOH.
- 2 Disconnect the manifold from the Retentate valve block and reconnect the ordinary outlet tubing.
- 3 After the System sanitization method, repeat the method using Milli-Q water (or equivalent) or buffer instead of sanitization solution.
- 4 Empty the system rinsing bottles and fill them with rinsing solution, 20% ethanol in 10 mM NaOH.
- 5 Replace the stirrer with a new aseptic one.

Changing reservoir size in UNICORN

The mounting of an alternative reservoir is described in *ÄKTAcrossflow Instrument Handbook*. However, it is also necessary to change the settings in UNICORN.

- 1 In the **System Control** of UNICORN, select **System:Settings**.
- 2 Select **Specials** as Instructions and scroll down to “Reservoir Size”.



- 3 Select **Size** of reservoir and click **OK**.

